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<p>(54) Title: PHARMACEUTICAL CONJUGATES OF GLYCOPROTEINS AND INSOLUBLE CARRIERS (57) Abstract A pharmaceutical conjugate of an insoluble carrier and a glycoprotein linked to the carrier via its carbohydrate moiety.</p>		

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PHARMACEUTICAL CONJUGATES OF GLYCOPROTEINS AND
INSOLUBLE CARRIERS

Field of the Invention

This invention relates to pharmaceutical conjugates, in particular those comprising two active agents, and to their production and use.

5 Background of the Invention

Pharmaceutical conjugates and their production are described in WO-A-98/17319. In particular, conjugates of albumin microparticles linked by means of a spacer to a glycoprotein, such as Factor VIII or fibrinogen, are described. Their production depends on the presence of functional SH groups on albumin, and functional
10 amine groups on the protein. It is recognised that retention of activity is an important parameter.

Summary of the Invention

The present invention is based on the utility of such conjugates, wherein the linking is *via* the carbohydrate moiety of the glycoprotein. In this way, the function of
15 the active protein moiety may be retained after binding the glycoprotein to the carrier.

Description of the Invention

The illustrative glycoprotein is, for example, fibrinogen or Factor VIII. Other suitable compounds are blood coagulation factors, proteins of the coagulation cascade, thrombolytic agents, antibodies or α -1 antitrypsin. If desired, another active agent may
20 be bound, covalently or by adsorption; see WO-A-98/17319 and also WO-A-99/25283.

By means of the present invention, glycoproteins such as FVIII or fibrinogen may be bound to microcapsules *via* a spacer. More specifically, the invention utilises the fact that a carrier such as HSA has free, SH, NH₂ or COOH groups, with which a bifunctional compound can react, the bifunctional compound having one group that may be selectively
25 reactive with the glycoprotein to be conjugated.

By virtue of the invention, controllable cross-linking can be achieved due to the specificity of one of the linking groups for the functional group available on on the carrier. Controllable cross-linking is one important aspect of the present invention, since it may have a direct bearing on the activity of the attached molecule.

30 The spacer can include enzyme-cleavable peptides, acid or alkali-labile bonds and be of variable length, depending on the requirements of the application. The length of

the spacer may be another important aspect of this invention, as it may determine the conjugate's ability to target receptors, such as fibrinogen to GPIIb/IIIa. Suitable spacer lengths are 10 to 600 nm, e.g. 20 to 400 nm.

A product of the invention may have utility as a platelet substitute. Such a product comprises an insoluble carrier, e.g. stabilised albumin, to which fibrinogen is bound, essentially without loss of the fibrinogen's activity.

This invention provides, *inter alia*, pure, robust, therapeutically-acceptable, platelet substitutes. Purity may be embodied in the absence of chemical cross-linker and/or surfactant. They are suitable for use in the treatment of thrombocytopenia.

It is an additional feature of the invention that, because fibrinogen acts as a targeting agent, products of the invention may usefully have other bound active agents. Such agents will be chosen with regard to the site of action, usually a wound or other bleeding locus, and to the nature of the problem that is addressed.

The carrier that is used in the invention is preferably produced by spray-drying, under conditions that allow good control of particle size and size distribution. For example, the preferred size is up to 6 μm , e.g. 1 to 4 μm , in order that the particles can pass through capillaries.

Suitable materials and procedures, and also methods for stabilising the microparticles, by heat or by chemical cross-linking, are fully described in WO-A-92/18164, WO-A-96/15814 and WO-A-96/18388, the contents of which are incorporated herein by reference. As explained in the latter publication, the conditions that are described do not affect functional groups, such as the thiol groups in albumin, which therefore remain available for reaction with biological molecules.

The microparticles used in this invention may have the physical characteristics described in the two publications identified above, e.g. being smooth and spherical, and containing air. In order to obtain insoluble, cross-linked microcapsules, the spray-dried product may be reacted with a chemical cross-linking agent. However, heat and γ -irradiation are preferred, and may also sterilise the dry powder products.

By providing a combination of, say, fibrinogen and Factor VIII, the products of the invention may be useful in the treatment of haemophilia. In addition, or as an alternative, to the use of a thrombolytic drug such as urokinase, blood clots may be

treated by the use of ultrasound. For this purpose, the air-containing microcapsules of this invention are especially suitable.

A bifunctional compound (say, Y^1-Y-Y^2) may be used in the invention, to link the carrier and the glycoprotein. Thus, for example, Y^1 is thiol-reactive.

5 The hydrazide method of crosslinking may be applied for the attachment of any moiety that possesses a carbohydrate functionality to HSA microcapsules. Specific examples include the γ -chain of fibrinogen, GB1b and Factor VIII.

 In order to attach the carbohydrate moiety to HSA microcapsules, the carbohydrate clusters should be subjected to a mild oxidation, e.g. with sodium
10 periodate. This would cleave the cis-diol component of the sugar ring to yield reactive aldehydes.

 The invention preferably utilises the hydrazide concept, as part of a heterobifunctional crosslinker. This linker possesses the hydrazide functionality at one end and be separated from a pyridyldithio group ($S-SC_5H_4N$) either by a simple carbon
15 chain length, or a more complex aromatic structure. The hydrazide group reacts with aldehyde groups on the periodate-treated γ -chain giving rise to a covalent hydrazone link. The pyridyldithiol group participates in a disulfide exchange reaction with the free thiol, Cys34, on HSA microcapsules.

 The carbohydrate attachments which contribute to both the beta and gamma
20 chains within the fibrinogen structure make ideal sites for crosslinking. Each oligosaccharide chain contains eleven monosaccharide units in total, comprising a combination of N-acetylglucose, mannose, galactose and N-acetylneuraminic acid (sialic acid) residues. The carbohydrates are linked through an asparagine residue on both fibrinogen chains. The carbohydrate structure of the gamma chain of human fibrinogen
25 is shown in Figure 1.

 A crosslinking spacer based on the hydrazide functional group has been developed for the covalent binding of fibrinogen to HSA microcapsules.

 In order to covalently bind the crosslinker to fibrinogen, modification of the terminal sialic acid residue is required. Selective periodate oxidation of the diol present
30 in the sugar side chain results in the formation of an active aldehyde. The sodium periodate concentration and the reaction temperature can be controlled to determine

whether sialic acid diols alone are cleaved or whether oxidation is non-selective. Further incubation with a hydrazide functional group results in hydrazone formation. Addition of sodium cyanoborohydride causes reduction and stabilisation of the hydrazone bond. This avoids the need for an activation step using 1-ethyl-3,3-dimethylaminopropylcarbodiimide (EDC).

Figure 2 shows a reaction method for the covalent attachment of PDPH to fibrinogen, starting from NeuAc residue (sialic acid). } = hydrazone.

The fluorescent label fluorescein-5-thiosemicarbazide (FTSC) has been used to establish the success of the crosslinking reactions involving both fibrinogen and HSA microcapsules. The carbazide moiety is similar to, and reacts in the same manner as, the hydrazide group contained within the crosslinker.

3-(2-pyridyldithio)propionylhydrazide (PDPH) is the preferred crosslinker. It can participate in both hydrazone formation with fibrinogen and a free thiol reaction with HSA microcapsules. The disulphide bond of the hydrazone-derivatised fibrinogen breaks and undergoes a free thiol exchange with the Cys-34 residue on the HSA microcapsules to form a second disulphide bond. This causes the release of pyridyl-2-dithione, the presence of which can be detected by absorbance at 343nm.

This crosslinking technology can be extended to include the attachment of isolated gamma chain, which had retained its glycosylation, Factor VIII and other glycoproteins.

In order to enhance the binding of fibrinogen (by way of example) via the spacer onto the microcapsules, the free thiol content of the microcapsules may be increased using Traut's reagent (2-iminothiolane). This reagent modifies the ϵ -amino groups of lysine into thiol groups, resulting in an increase in the number of free thiols available to bind with the spacer and thus fibrinogen.

Increased loading may also be achieved by cross-linking thiol-containing amino-acids or peptides to the microcapsules prior to attaching the protein of interest (e.g. cysteine, reduced glutathione). Chemical linkers such as iminothiolane can be used to introduce (as well as increase the number of) thiol groups. Other proteins could be used to produce microcapsules if thiol groups were added to their surface, as an alternative to using the inherent properties of HSA (i.e. free thiol groups).

As indicated above, products of the invention containing fibrinogen may act at the site of tumours. Therefore, they may be used in tumour therapy, e.g. by linking a cytotoxic agent by the particular method of this invention or by the methods described in WO-A-96/18388. Suitable cytotoxic agents include methotrexate, doxorubicin, 5 cisplatin or 5-fluoro-2'-deoxyuridine.

The targeting of drugs to tumour cells may be achieved using products of the invention as vehicles reacting directly with the cells or by participating in the aggregation and deposition of fibrin at the site of cell adhesion.

Products of this invention may be loaded with cytotoxic agents or a combination of cytotoxic and targeting agents. They may then be used to target the disseminated 10 tumour cells in the circulation, by specific interactions with the cell glycoprotein receptors (seek and destroy) or by participation in the platelet aggregation process at the site of adhesion. In both cases, the cytotoxic drug is concentrated at the site of the invading tumour cells.

15 Alternatively, tumour aggregation may be inhibited in the circulatory system, or even at the site of adhesion, by coating the tumour cell surface with products of the invention, and blocking the sites/mechanisms that activate platelets. This would then allow the body's natural defence mechanisms to facilitate the removal of the tumour cells.

Products containing, for example, the GPIb receptor (interacts with von 20 Willebrands factor) or receptors for collagen or other sub-endothelial matrix components may also be delivered, to potentially block the binding sites for tumour cells by coating the sub-endothelial matrices. The product should still allow an interaction with platelets at the site of a wound, but should also restrict the invasion of vascular wall by any immobilised tumour cell.

25 An important advantage of the present invention is that the activity of fibrinogen (or other glycoprotein) can be substantially retained. The content of active fibrinogen can be determined by known procedures; see WO-A-98/17319.

A platelet substitute of the invention usually comprises at least 0.01%, preferably at least 0.015%, more preferably at least 0.02%, and most preferably at least 0.025%, 30 active fibrinogen. The amount of fibrinogen should not be too great, in order to avoid aggregation, e.g. up to 1, 1.5, 2 or 2.5%. Of the fibrinogen content, it is desirable that

at least 50%, preferably at least 70%, more preferably at least 90%, should be active. This can be determined with respect to the total content of fibrinogen, which again can be measured by method such as ELISA. Total fibrinogen may also be determined by radio-labelling, e.g. with ^{125}I , and counting, by conventional procedures.

5 The fibrinogen may be blood-derived, transgenic or recombinant, full-length or any active fragment thereof. Fragments are disclosed, *inter alia*, by Collier *et al*, J. Clin. Invest. 89:546-555 (1992).

 For use as a therapeutic agent, a product of the invention may be administered as is, or mixed with any suitable carrier known to those of ordinary skill in the art. The
10 amount of the product administered will be determined largely to the severity of the wound or other condition to be treated. A typical dosage may be 1.5×10^9 microcapsules per kg body weight.

 The following Examples illustrate the invention.

 The fibrinogen used in the Examples was a full-length, blood-derived,
15 commercially available product that had been doubly virally-inactivated.

 HSA microcapsules used in the Examples were prepared by spray-drying and were then stabilised by heating, as described in WO-A-9615814. The microcapsules were sunk with 1% Tween 80 and washed extensively with PFPW to remove Tween 80 and excipient prior to use.

20 PFPW = pyrogen-free purified water.

 DTNB = 5,5-dithiobis(2-nitrobenzoic acid).

 Free thiol content was measured using the Ellman assay with DTNB. This reagent participates in a thiol exchange mechanism with any free thiols present on the protein under examination, and releases (TNB) which can be measured at 412 nm using
25 UV/VIS spectrophotometry.

 Sodium phosphate buffer pH 7.0 was used to make the stock fibrinogen solution. Two concentrations, 0.1M and 0.01M, were compared. The reaction of fibrinogen with 1mM sodium periodate at 0°C was quenched with glycerol after 30 minutes. A control was set up for both reactions in which an equal volume of reaction buffer was added in
30 place of sodium periodate.

The use of 0.001M sodium periodate resulted in the formation of 68.8nmols of aldehyde (74.1% yield). Increasing this concentration to 0.002M caused other sugar residues besides sialic acid to be oxidised during the reaction as the calculated percentage +yield gave a value greater than 100%.

5 The conditions required for periodate oxidation were established as 0.001M sodium periodate at 0°C for 30 minutes followed by glycerol quenching.

FTSC was added to the samples in a 10 molar excess with respect to the number of moles of aldehydes per mole of fibrinogen present. The reaction was carried out in the absence of light. An aliquot was removed after 30 minutes and the remainder after 70
10 hours. See King *et al*, Biochem. (1986) 25:5774-9, and Hermanson, Bioconjugate Techniques, 1st ed., Acad. Press, USA (1996); these studies suggest that hydrazone formation can take 30 minutes or 70 hours for a 60% yield at pH 6.0, under certain conditions.

Excess FTSC was removed by purification of the sample through a PD-10 G25
15 sephadex column. After elution using 0.1M sodium phosphate, pH 7.2/0.1M sodium chloride buffer, the samples were loaded onto a reducing SDS-PAGE gel to determine whether the formation of the semicarbazone linkage had been successful.

Analysis under UV illumination allowed fluorescence to be observed in both the 70 hour control and reaction samples, but not in those removed after 30 minutes. The
20 positive results obtained from the control suggest that a degree of adsorption occurs over a long reaction time.

Reaction conditions for semicarbazone formation comprise a 2 hour reaction time at a temperature of 37°C. Further, results show that a greater yield is obtained at pH 6.0, although the pH 5.8 values are very similar after 2 hours. Addition of salt and increase
25 in pH may be used to eliminate buffer change during fibrinogen purification.

The addition of glycerol may be used to quench the oxidation reaction.

An activity test with thrombin performed on the purified control and reaction samples gave a positive result. The clot formation of the reaction sample was equivalent to a control which had not been treated with sodium periodate.

30 A marked increase in the absorbance values obtained using purification as a quenching method suggests that glycerol does have an inhibitory effect on fluorescence

as well as clot formation. Column purification was, therefore, recommended for future reactions.

In an attempt to quantify the semicarbazide binding onto fibrinogen, an FTSC standard curve was produced, from 0 to 40 nmoles. The reaction sample appeared to
5 yield double the concentration of bound semicarbazide than the control.

The number of moles of semicarbazide bound per mole of fibrinogen were calculated by firstly determining the number of moles of fibrinogen contained in the reaction sample. The number of nmoles of semicarbazide present were obtained from
10 reading the sample absorbance against the FTSC standard curve. The control sample value was then subtracted from that of the reaction sample and divided by the number of moles of fibrinogen. The resultant value was 2.516 moles semicarbazide/ mole fibrinogen. The theoretical value for a complete reaction is 8 moles/mole.

Addition of the mild reducing agent sodium cyanoborohydride (NaCNBH_3), post-hydrazone formation, stabilises the product via Schiff base reduction. An equal volume
15 of NaCNBH_3 was added to a reaction sample and incubated at 0°C for 40 minutes. After purification the absorbance at 492nm was recorded. A slight decrease in the readings was observed but this was thought to be due to the shift in excitation maximum. The new maximum was identified as 495nm on wavelength scanning of the sample. The conversion of the $\text{N}=\text{C}$ double bond of the hydrazone to a single bond during reduction
20 resulted in the change of excitation maximum.

The success of the free thiol reaction was determined by reacting PDPH with both HSA solution and microcapsules. The absorbance at 343nm was used to detect the presence of pyridyl-2-dithione released as a side product from the reaction.

HSA solution (1ml at 138mg/ml, 2090nmoles) in 20 molar excess was incubated
25 with PDPH (23.9 μg , 104nmoles) for one hour, an aliquot was removed and the absorbance at 343nm measured. Dithiothreitol (DTT) was added to the remainder of the sample. Any remaining PDPH would react with the DTT releasing the pyridyl-2-dithione moiety, but no increase in absorbance was observed. Therefore, it was assumed that no unreacted PDPH remained and that the reaction with HSA solution was complete.

The crosslinker was also reacted with HSA microcapsules as above. Microcapsule slurry concentrations of both 50 and 100mg/ml were used. The PDPH was dissolved in dichloromethane (DCM) at 100mg/ml microcapsule concentration.

The conditions required for production of derivatised fibrinogen were defined as
 5 0.001M sodium periodate in 0.1M sodium phosphate pH 5.8 for periodate oxidation and two column purification steps utilising 0.1M sodium phosphate pH 7.2/0.1M sodium chloride for elution. Addition of crosslinker in DCM at high concentration was followed by reaction at 37°C for 2 hours.

In an attempt to take the free thiol exchange reaction to completion in one hour,
 10 the free thiol content of the microcapsules were increased using Traut's reagent. Two different molar equivalents were compared, giving free thiol values of 1.6 and 4 nmoles SH/nmole HSA. A sample count was obtained post-reaction. This ensured that exactly 4 mg was delivered to the Free Thiol Assay, as a large percentage of microcapsules were lost during the extensive washing required to remove the excess Traut's reagent. The
 15 modified microcapsules were then reacted with PDPH and the absorbance recorded as before. The results are given in Table 1.

Table 1

Number molar equivalents	Volume added (4mg)/ μ l	nmoles SH / nmoles HSA	T1 Absorbance / 343nm	T2 Absorbance / 343nm
0 (control)	64.9	0.245	0.0182	0.0838
5	137.9	1.59	0.0229	0.0394
40	119.7	3.65	0.0362	0.0470

25 The absorbance readings indicate that the reaction is nearer completion after one hour when the free thiol content of the microcapsules is increased. The margin of increase in absorbance once DTT is added is dramatically reduced using both 5 and 40 molar equivalents of Traut's reagent when compared to the control sample. Although the 40 molar equivalent sample gave a higher yield of free thiol exchange, the use of 5
 30 molar equivalents were chosen as a fewer number of washes were required to remove excess reagent. A smaller percentage of microcapsules are also lost as a result.

From these experiments, an increase in the microcapsule free thiol content is preferred, for greater reaction success. The use of 5 molar equivalents of Traut's reagent, to give a free thiol of 1.6 nmoles SH per nmole HSA, was defined as the reaction conditions.

5 Reaction between fibrinogen and PDPH results in the transformation of a hydrazide moiety into a hydrazone. The hydrazide moiety, which is similar to the semicarbazide used above, contains a carbon-oxygen double bond in the reactive group. This replaces the carbon-sulphur double bond which appears in the semicarbazide reactive group. The data obtained for the model semicarbazide indicated that the resultant
10 absorbance values of pH 5.8 and 6.0 were comparable. Therefore, initial investigations into the binding of PDPH to fibrinogen used 0.1M phosphate pH 5.8 reaction buffer.

PDPH-derivatised fibrinogen was performed using the conditions summarised above.

Microcapsules (100mg) were sunk in 1% Tween 80 and washed in 10mM sodium
15 phosphate pH 6.0 to remove excipients. From a 10mg/ml stock solution of Traut's reagent, a volume corresponding to 5 molar equivalents was added. The total reaction volume was made to 2ml with reaction buffer, 10mM sodium phosphate pH 6.0, and incubated at room temperature for one hour. The numerous post reaction washes required, retained only 63 mg of microcapsules. The samples were then reconstituted in
20 2ml of buffer to give a final concentration of 31mg/ml and reacted with 1mg PDPH-derivatised fibrinogen for one hour at room temperature. The resultant solution was then washed in reaction buffer to remove any excess fibrinogen and slide tests performed to assess activity. The control sample remained inactive to thrombin after one minute; however, aggregation of the reaction sample was complete in 1-4/5 seconds.

25 In summary, the use of the fluorescent label, FTSC in these investigations has led to proof of concept being established as well as an estimation of the percentage yield of semicarbazone/hydrazone formed. It was also used successfully to monitor the free thiol exchange which occurs between HSA microcapsules and the derivatised fibrinogen during covalent attachment. Once the reaction conditions had been defined using the
30 model, the reactions were repeated employing the chosen crosslinker, PDPH. The

covalent attachment of fibrinogen to HSA microcapsules was achieved and this was reflected in the positive aggregation test results.

The current conditions for the crosslinking of fibrinogen to HSA microcapsules are summarised in Table 2.

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Table 2

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Item	Description
Step 1 Fibrinogen Sodium Periodate Reaction Buffer and Conditions Purification Elution Buffer	5mg FS-1-004-4 (1ml at 5mg/ml) 218µg (21.8µl at 10mg/ml) 0.1M Sodium Phosphate pH 5.8 at 0°C for 30 minutes 0.1M Sodium Phosphate pH 7.2/ 0.1M Sodium Chloride
Step 2 Fibrinogen PDPH Reaction Time and Conditions	5mg (1.42mg/ml after purification above) 0.48 mg in DCM (20µl at 24mg/ml) 2 hours at 37°C
Step 3 HSA microcapsules 2-Iminothiolane (Traut's reagent) Reaction and Post-Reaction Buffer Reaction Time and Conditions	200mg mannitol formulation 2.08mg, 5 molar equivalents (208µl at 10mg/ml) 10mM Sodium Phosphate pH 6.0 1 hour at RT
Step 4 PDPH derivatised Fibrinogen (from Step 2) HSA microcapsules (from Step 3) Reaction Time and Conditions	1mg (1ml at 1mg/ml in elution buffer) 62mg (2ml at 31mg/ml, unformulated) 2 hours at RT

CLAIMS

1. A pharmaceutical conjugate of an insoluble carrier and a glycoprotein linked to the carrier *via* its carbohydrate moiety.
- 5 2. A conjugate according to claim 1, wherein the carrier is a protein having functional groups selected from SH, NH₂ and COOH.
3. A conjugate according to claim 2, wherein the carrier is human serum albumin.
4. A conjugate according to any preceding claim, wherein the carrier is in the form of microcapsules.
- 10 5. A conjugate according to any preceding claim, wherein the glycoprotein is Factor VIII or fibrinogen.
6. A product according to any preceding claim, wherein the linker is derived from a bifunctional reagent reactive with the carbohydrate moiety or CHO obtainable from said moiety, and also with a functional group on the carrier.
- 15 7. A conjugate according to claim 6, wherein the reagent is reactive with CHO.
8. A conjugate according to claim 7, wherein the reagent is a hydrazide.
9. A conjugate according to claim 8, wherein the reagent is PDPH.

1/2

Figure 1

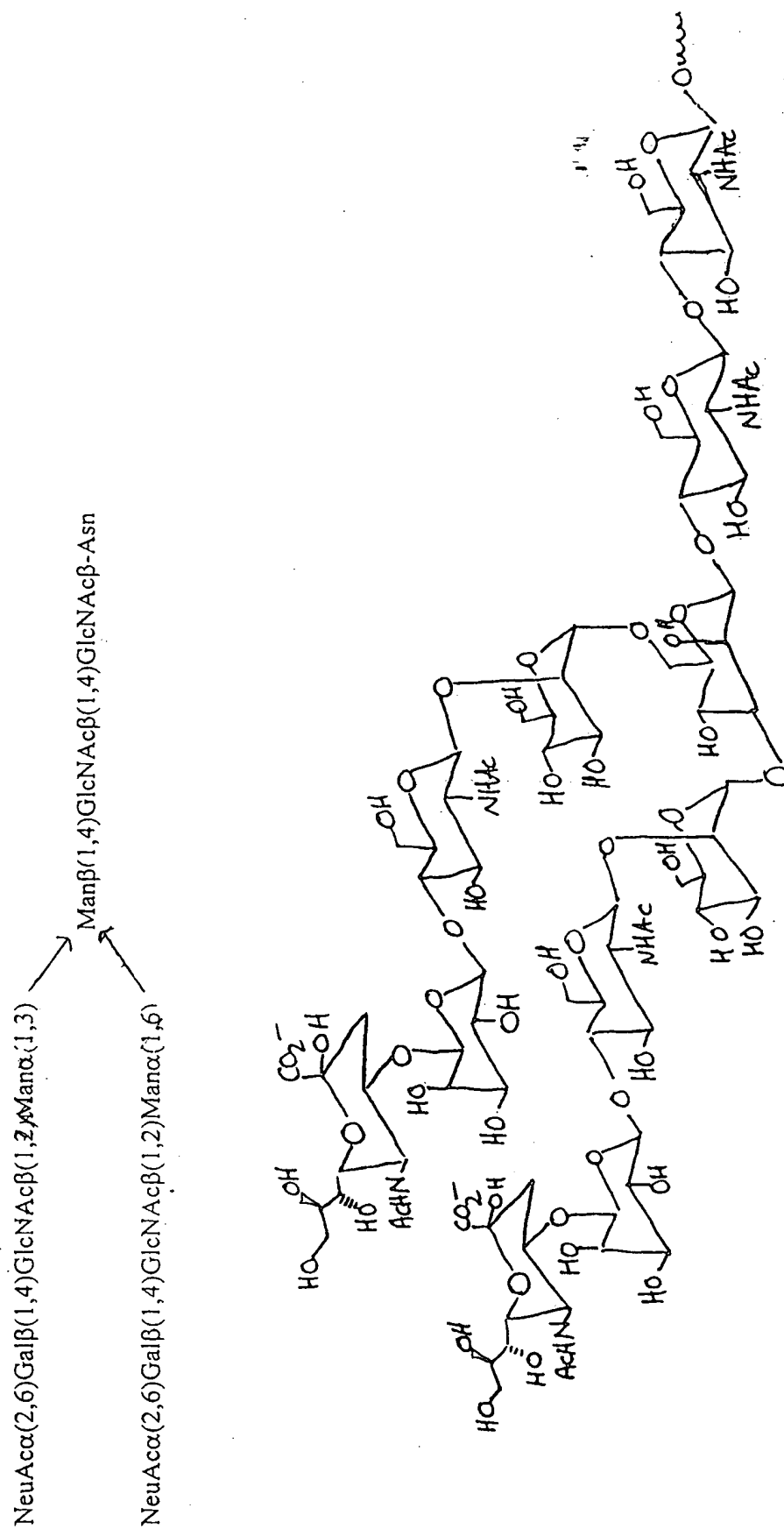
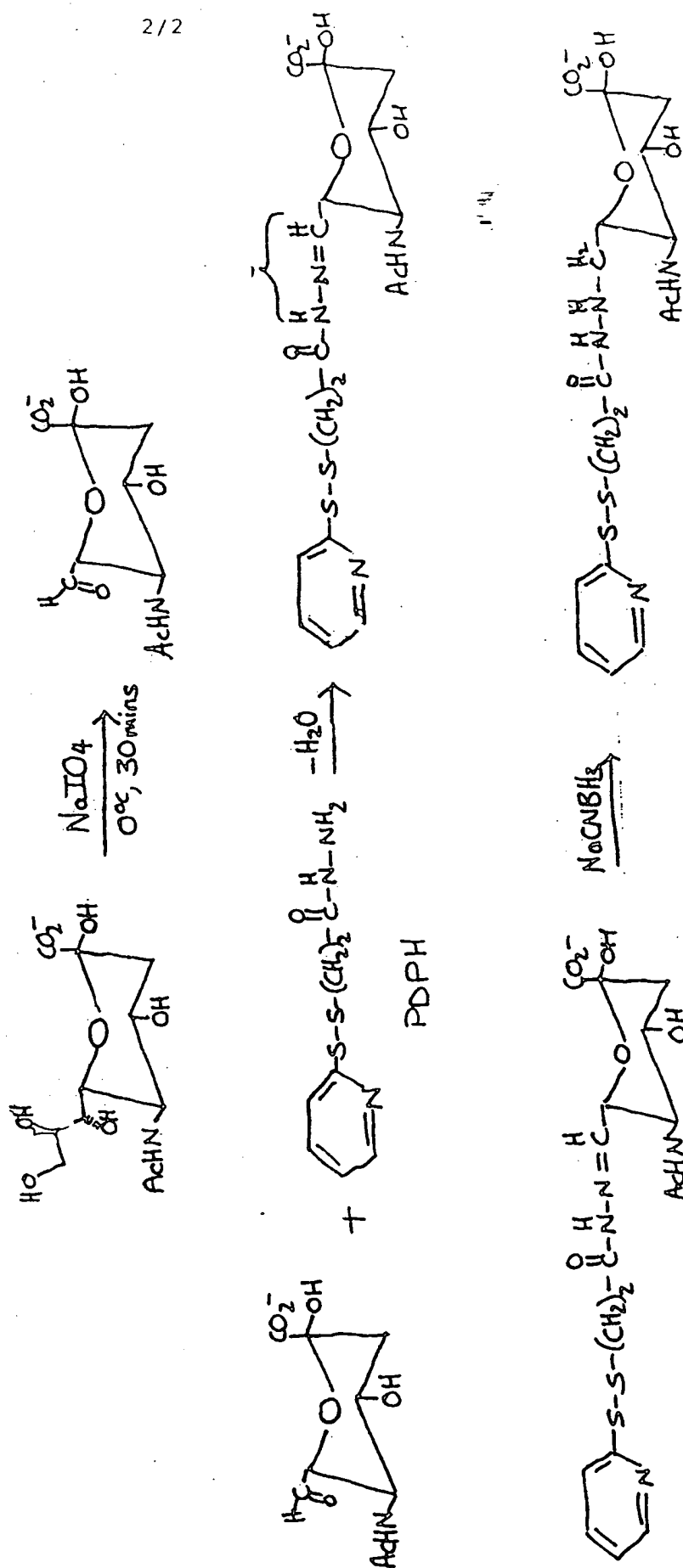


Figure 2



INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/03803

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 17319 A (ANDARIS LTD) 30 April 1998 (1998-04-30) cited in the application abstract page 2, line 21 - page 3, line 2 page 3, line 1 - line 2 page 4, line 4 - line 8 page 5, line 20 - line 24 page 6, line 12 - line 14 claims 1-26	1-9
X	US 5 308 617 A (HALLUIN ALBERT P) 3 May 1994 (1994-05-03) abstract column 4, line 12 - line 27 column 6, line 40 - line 65 claims 1-14	1,2,6-9
	-/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Name and mailing address of the ISA

European Patent Office, P.B. 5018 Patentaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Taylor, G.M.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>QUASH, G.A. ET AL.: "Diagnostic and Therapeutic Procedures with Haptens and Glycoproteins (Antigens and Antibodies) Coupled Covalently by Specific Sites to Insoluble Supports"</p> <p>TARGETED DIAGNOSTICS AND THERAPY, vol. 2, 1989, pages 155-186, XP000882639</p> <p>introduction</p> <p>Section II.2.A</p> <p>Section II.4</p> <p>Section IV.C</p> <p>Section V.B</p>	1,2,4, 6-8
P,X	<p>WO 99 25383 A (HARRIS ROY ;CHURCH NICOLA JANE (GB); QUADRANT HEALTHCARE UK LIMITE) 27 May 1999 (1999-05-27)</p> <p>abstract</p> <p>page 1, line 12 - line 25</p> <p>page 2, line 6 - line 29</p> <p>page 3, line 3 - line 7</p> <p>page 4, line 20 - line 25</p> <p>page 5, line 18 - line 31</p> <p>page 6, line 17 - line 27</p> <p>claims 1-13</p>	1-9
A	<p>WO 96 18388 A (ANDARIS LTD ;JOHNSON RICHARD ALAN (GB); SUTTON ANDREW DEREK (GB)) 20 June 1996 (1996-06-20)</p> <p>cited in the application</p> <p>abstract</p> <p>page 4, line 3 - line 22</p> <p>page 7, line 35 -page 8, line 8</p> <p>claims 1-14</p>	1-9

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Information on patent family members

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